IDENTIFICATION OF SUPPLEMENTS THAT ENHANCE THE RECOVERY OF *LISTERIA MONOCYTOGENES* ON MODIFIED VOGEL JOHNSON AGAR

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ABSTRACT

Modified Vogel Johnson agar (MVJ) has high selectivity for Listeria monocytogenes*; however, it does not permit the repair and subsequent colony formation of injured cells. By the addition of 0.4% Tween 80, 25 mL/L bovine fetal serum, or 50 mL/L egg yolk tellurite enrichment (50%), the ability of MVJ to detect heat injured* L. monocytogenes *(Scott A strain) was increased approximately 100-fold without loss of selectivity.*

INTRODUCTION

Sublethal food processing can lead to injury rather than death of microorganisms that may be present. Under the right conditions, injured microorganisms present in food products may repair and start to grow. When selective media are used to determine bacterial numbers, injured microorganisms are not normally detected since most of the compounds used as selective agents prevent the repair and growth of injured cells (Smith and Palumbo 1982). Underestimating the count of a pathogenic bacterial species due to failure to detect injured cells can lead to a potentially hazardous food product.

Injury has been demonstrated in *Listeria monocytogenes* (Beuchat *et al.* 1986; Dallmier and Martin 1988; Golden *et al.* 1988; Smith and Archer 1988); but little work has been done on the repair of injured *L. monocytogenes*. Buchanan and his coworkers (1987) have developed a *Listeria*-selective medium (Modified Vogel-Johnson agar; MVJ) based on Vogel-Johnson agar containing potassium

tellurite, moxalactam, bacitracin, and sodium nalidixate. While the selectivity of MVJ is quite good and colonies of *Listeria* easy to detect because of the black color developed on reduction of tellurite, the medium is probably inhibitory to the repair of injured cells due to its selective agents. In the current study, we have used *L. monocytogenes*, Scott A strain as a representative strain of the species to determine the effects of various compounds on the repair and growth of heat-injured cells, and developed a modified formulation that enhances greatly the recovery of sublethally stressed *Listeria*.

METHODS AND MATERIALS

Microorganisms

Listeria monocytogenes, Scott A strain, was maintained in brain heart infusion broth (BHI; Difco) stored at 5 °C. One-hundred mL of BHI containing additional glucose (final glucose concentration, 0.5% w/v) was inoculated with *L. monocytogenes* and incubated on a rotary shaker (200 rpm) at 37 °C for 18–20h. Cells were harvested by centrifugation, washed twice with sterile distilled water and resuspended in 5 mL sterile distilled water. *Escherichia coli* 11602 and *Staphylococcus aureus* 196E were maintained in tryptic soy broth (Difco) stored at 5 °C. *E. coli* and *S. aureus* were inoculated into BHI and incubated on a rotary shaker (200) rpm at 37 °C for 18–20 h, but were not washed.

Injury Procedure

To determine heat injury, a screw-cap 160 mL dilution bottle containing 20 mL sterile potassium phosphate buffer (pH 7.2; 0.1 M) was equilibrated to 52 °C and then 1 mL of washed *L. monocytogenes* was added to give 2–4 $\times$ 10^9 cells/mL buffer. The buffer-cell suspension was agitated (approximately 150 agitation/min) on a Burrell Wrist-Action Shaker for 2 h. At zero time and at 2 h, 0.1 mL of culture was removed from the bottle and added to 9.9 mL sterile 0.1% (w/v) Bacto peptone water and successive dilutions prepared. Using a spiral plater (Spiral Systems Instruments, Inc., Bethesda, MD), appropriate dilutions were plated into tryptose phosphate broth $\pm$2% agar (w/v) + 1% (w/v) Na pyruvate (TPBA + P), TPBA + 5% (w/v) added NaCl (TPBA + S), and onto MVJ with and without the addition of various compounds (Table 1). Plates were incubated at 37 °C and counted after 3 days.

Selectivity Studies

Selectivity of MVJ, MVJ + 0.4% Tween 80 or MVJ + bovine fetal serum was analyzed by plating (using the spiral plater) dilutions of unwashed, noninjured *L. monocytogenes* Scott A strain, *E. coli* 11602 or *S. aureus* 196 onto the

TABLE 1.
THE EFFECT OF VARIOUS ADDITIVES TO MODIFIED VOGEL-JOHNSON AGAR (MVJ) ON RECOVERY OF HEAT-INJURED *L. monocytogenes*

Medium	n[a]	Log 10 Viable Cells/ml After holding at 52°C for 0 Min	120 Min
TPBA + P	26	9.61 (0.13)[b]	8.45 (0.38)
MVJ + Tween 80 (0.4 %)	15	9.47 (0.25)	7.41 (0.49)**[c]
MVJ + bovine fetal serum (25 ml/l)	13	9.53 (0.16)	7.37 (0.62)**
MVJ + EY tellurite enrichment (50 ml/l)	8	9.52 (0.19)	7.20 (0.54)**
MVJ + egg yolk (50 ml/l)	6	9.50 (0.19)	6.48 (0.39)*
MVJ + phosphatidylcholine (1.0%)	6	9.36 (0.13)	6.43 (0.51)*
MVJ	26	9.46 (0.20)	5.96 (0.80)
MVJ + soybean lecithin (4.0%) + Tween 80 (0.4 %)	6	9.21 (0.16)	5.68 (0.42)*
TPBA + S	26	9.57 (0.15)	5.34 (0.48)

[a] *n* = number of replicates.
[b] The mean is followed by standard deviation of the mean in parenthesis.
[c] Supplemented media listed with * were not significantly different from MVJ at the 99% confidence level; those listed with ** were significantly different from MVJ at the 99% confidence level.

abbreviations: TPBA + P = tryptose phosphate broth + 2% agar + 1% Na pyruvate; TPBA + S = tryptose phosphate broth + 2% agar + 5% NaCl; MVJ = modified Vogel Johnson agar.

agars and incubating at 37 °C for two days. In order to assess the growth of natural microflora on these agars, raw hamburger, raw pork sausage, frankfurters, and raw goat's milk were purchased from a local retail market. Fifty g of each food was aseptically removed from each package and added to 200 mL of sterile peptone water; the samples were vigorously mixed using a Stomacher 400 (A.H. Thomas Co.). One tenth mL of each dilution (1/5 to 10^7) was placed on the surface of agar media and spread using a sterile glass spreader. When plates were dry, they were incubated at 37 °C for 3 days. Gram and catalase reactions were determined on major colonial types.

Media and Chemicals

All bacteriological media and agar were obtained from Difco Labs (Detroit, MI). Sodium pyruvate, L-α-phosphatidylcholine from dried egg yolk (approximately 60% phosphatidylcholine), polyethylene sorbitan mono-oleate (Tween 80), sodium nalidixate, bacitracin, and potassium tellurite were obtained from Sigma Chemical Co. (St. Louis, MO) and disodium moxalactam was obtained from Eli Lilly & Co. (Indianapolis, IN). Egg yolk emulsion (exp. date 6-89) was obtained from Remel (Lenaxa, KS) and EY tellurite enrichment obtained from Difco Labs (obtained 8/81) were stored at 5 °C. Liquid soybean lecithin was obtained from Fearn Natural Foods, Milwaukee, WI. Fetal bovine serum was obtained from Flow Labs, Inc. (McLean, VA).

Statistics

Data was analyzed by one-way analysis of variance using the Ecstatic (Someware in Vermont, Montpelier, VT) and Number Cruncher Statistical System (J.L. Hintze, Kaysville, UT) statistical software programs.

RESULTS AND DISCUSSION

In order to determine the number of heat injured *L. monocytoenes,* a two-media plating system was used: TPBA + P on which both injured and noninjured cells produce colonies and TPBA + S on which only noninjured cells grew. Injured cells can repair on TPBA + P but can not repair in the presence of the selective agent NaCl in TPBA + S (Smith and Archer 1988). Subtracting the count on TPBA + S from that on TPBA + P (after 2 h heating at 52C) gave an estimation of injury.

Initial experiments indicated that addition of sodium pyruvate (1–3%, autoclaved with agar medium) or the enzyme, catalase (a filter-sterilized solution of Worthington lyophilized catalase was added aseptically to tempered agar to give 0.1% final concentration), to MVJ did not lead to increased counts when heat injured cells were plated (data not shown). It has been shown that the addition of pyruvate or catalase to selective media used for the isolation of other microorganisms that may have been subjected to sublethal processing conditions enhanced colony formation by injured cells (Smith and Palumbo 1982). Both catalase and pyruvate decompose hydrogen peroxide which is particularly deleterious to injured cells (Martin *et al.* 1976; Brewer *et al.* 1977; Humphrey 1988). It is not known why pyruvate or catalase addition was not effective with *L. monocytogenes*. These results agree with Smith and Archer (1988) who demonstrated that the addition of pyruvate to modified McBride medium did lead to repair and colony formation by heat-injured cells of *L. monocytogenes.*

None of media were inhibitory to uninjured cells (Table 1), i.e., the zero time counts were approximately the same as that on TPBA + P. After 2 h of heating at 52 °C, the count on MVJ by itself was only slightly better than that on TPBA + S indicating that MVJ did not support repair and colony formation by injured *L. monocytogenes*.

The addition of 0.4% Tween 80, 25 mL/L bovine fetal serum, or 50 mL/L Bacto EY tellurite emulsion to MVJ significantly (at 99% confidence level) improved colony formation by injured cells on MVJ (Table 1). Addition of Remel egg yolk emulsion or phosphatidylcholine to MVJ improved recovery of injured cells; however, the differences were not significant from MVJ alone at the 99% confidence level. The reason for the difference between the two sources of egg yolk emulsion is not readily apparant, but could reflect differences in age or source of the products or the presence of tellurite in EY tellurite emulsion (when EY tellurite emulsion was added to MVJ, the tellurite level of MVJ was reduced in order to keep the tellurite level comparable to MVJ without the addition). Since Tween 80 and bovine fetal serum proved more effective, this question was not pursued. Addition of soybean lecithin was ineffective (Table 1). None of the supplements to MVJ led to complete recovery, i.e., counts equal to those found on TPBA + P at 120 min. of heating (Table 1), indicating that the inhibitory aspects of MVJ could not be completely overcome.

TABLE 2.
EFFECT OF ADDITIONS OF TWEEN 80 AND BOVINE FETAL SERUM TO MVJ ON RECOVERY OF UNHEATED *Listeria monocytogenes* SCOTT A. STRAIN, *Escherichia coli* 11602 AND *Staphylococcus aureus* 196E

	Log 10 Viable Cells/ml		
Medium	*L. monocytogenes*	*E. coli*	*S. aureus*
TPBA + P	9.65 (0.22)[a]	9.48 (0.15)	8.57 (0.060)
MVJ	9.54 (0.11)	< 1.30[b]	3.52 (0.57)
MVJ + Tween 80 (0.4%)	9.55 (0.06)	< 1.30	3.50 (0.10)
MVJ + bovine fetal serum (25 ml/l)	9.60 (0.10)	< 1.30	3.51 (0.06)

[a]The values given represent the mean of six different determinations and the numbers in parentheses represent standard deviation of the mean.
[b]Lower limit of detection.

abbreviations: TPBA + P = tryptose phosphate broth + 2% agar + 1% Na pyruvate; MVJ = modified Vogel Johnson agar.

TABLE 3.
EFFECT OF ADDITIONS OF TWEEN 80 (0.4%) AND BOVINE FETAL SERUM (25 mL/1) TO MVJ ON GROWTH OF MICROFLORA PRESENT IN CERTAIN FOODS

Food	Plating Medium	Dilution	# of Colonies on Plates	Major Microbial Types
Plated on Day of Purchase				
Hamburger	TPBA + P	10^4	56	G -, C + rods; G +, C - rods
	MVJ	$10^{1.7}$	2	G +, C + cocci
	MVJ + Tween 80	$10^{1.7}$	0	
	MVJ + BFS[c]	$10^{1.7}$	0	
Pork Sausage	TPBA + P	10^4	102	G +, C + cocci; G -, C+ rods
	MVJ	$10^{1.7}$	2	G +, C + cocci
	MVJ + Tween 80	$10^{1.7}$	3	G +, C + cocci
	MVJ + BFS	$10^{1.7}$	3	G +, C + cocci
Frankfurters	TPBA + P	10^6	55	G-, C + rods; G +, C - cocci
	MVJ	$10^{1.7}$	0	
	MVJ + Tween 80	$10^{1.7}$	1	G +, C + cocci
	MVJ + BFS	$10^{1.7}$	0	
Goats Milk (Raw)	TPBA + P	10^2	46	G +, C + cocci
	MVJ	$10^{1.7}$	0	
	MVJ + Tween 80	$10^{1.7}$	3	G +, C + cocci
	MVJ + BFS	$10^{1.7}$	0	

TABLE 3. continued

Plated after 4 Days Storage at 5°C

Hamburger	TPBA + P	10^6	110	G -, C + rods; G +, C - rods
	MVJ	$10^{1.7}$	2	G +, C + rods
	MVJ + Tween 80	$10^{1.7}$	2	G +, C + rods
	MVJ + BFS	$10^{1.7}$	0	
Pork Sausage	TPBA + P	10^4	54	G +, C + rods
	MVJ	$10^{1.7}$	0	
	MVJ + Tween 80	$10^{1.7}$	3	G +, C + cocci
	MVJ + BFS	$10^{1.7}$	4	G +, C + cocci
Frankfurter	TPBA + P	10^8	60	G +, C + rods; G +, C - rods
	MVJ	$10^{1.7}$	1	G +, C + cocci
	MVJ + Tween 80	$10^{1.7}$	0	
	MVJ + BFS	$10^{1.7}$	0	
Goats Milk (raw)	TPBA + P	10^2	69	G +, C + cocci
	MVJ	$10^{1.7}$	0	
	MVJ + Tween 80	$10^{1.7}$	0	
	MVJ + BFS	$10^{1.7}$	0	

abbreviations: G = *gram reaction; C* = catalase reaction; TPBA + P = tryptose phosphate broth + 2% agar + 1% Na pyruvate; MVJ = modified Vogel Johnson agar; BFS = bovine fetal serum.

The addition of Tween 80 or Bovine Fetal Serum was evaluated to determined the effect of supplementation might have on the selective behavior of MVJ (Table 2). *E. Coli* showed no growth on MVJ regardless of the addition and *S. aureus* was decreased 5 log units on MVJ with or without additions (as compared to TPBA + P). Plating of representative food samples indicated that supplementation with Tween 80 or bovine fetal serum did not detract from the ability of MVJ to suppress the natural microflora present in foods (Table 3). Thus, the addition of Tween 80 or bovine fetal serum did not decrease the selectivity of MVJ.

Use of automatic (laser) colony counters require a clear agar medium. Egg yolk gives a cloudy medium that makes it difficult to use even with a regular colony counter since the grids are difficult to see. Both Tween 80 and bovine fetal serum when added to MVJ give a clear medium through which the grids of a regular colony counter are visible as well as giving media that can be used with the automatic colony counter.

At the present time, the mechanism(s) by which bovine fetal serum, Tween 80, or egg yolk emulsion lead to repair and colony formation of injured cells when added to MVJ agar is unknown. Since the primary site of heat injury in bacteria is the bacterial membrane (Busta 1976; Hurst 1976; Smith and Palumbo 1982), it is possible that these compounds protect the injured cells against loss of cellular integrity or that the additives may be a source on building blocks to repair the damaged membrane. Alternately, serum, Tween 80, or egg yolk may bind some toxic moiety present in the bacterial medium. Whatever the mechanism may be, the data presented here indicated that the addition of bovine fetal serum, egg yolk, or Tween 80 to MVJ enhances the ability of this highly selective medium to isolate *L. monocytogenes* from foods under conditions when sublethal stress may be suspected.

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